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(54) **Sodium hyaluronate based ophthalmic formulation for use in eye surgery**

Ophthalmische Formulierung auf Natriumhyaluronatbasis zur Verwendung in der Augenchirurgie

Formulation ophthalmique à base de l'hyaluronate de sodium pour l'utilisation en chirurgie de l'oeil

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**EP 0 781 547 B1**

**Description**Field of the Invention

- 5 [0001] The present invention relates to an ophthalmic formulation, for use in eye surgery, comprising sodium hyaluronate, citrate, at least one antioxidant tolerated by the intraocular tissues and a phosphate buffer.
- [0002] Particularly, the present invention will comprise sodium hyaluronate having molecular weight from 1,000,000 to 2,000,000 Daltons.

10 Prior Art

- [0003] Sodium hyaluronate (NaHA) based solutions have already been used for some time in eye surgery.
- [0004] Loss of corneal endothelial cells and elevation in intra-ocular pressure (P.I.O.) after cataract operations with or without implantation of intraocular lenses are two negative consequences of such operations. Particularly, loss of
- 15 endothelial cells can cause serious problems since such cells do not regenerate.
- [0005] As for causes of elevation in pressure and mechanisms related with the loss of endothelial cells, various hypothesis have been suggested (Liesegang, T.J., 1990, Survey Ophthalmol., 34, 268-293). Nevertheless, the problems are still unsolved and the discovery of novel viscoelastic products able to limit or eliminate injuries resulting from operation, is still of great interest for safety of eye surgery.
- 20 [0006] Sodium hyaluronate of pharmaceutical purity has already been used in production of solutions for eye surgery and various methods of preparation of the polymer having molecular weight from about 150,000 to about 6,000,000 Daltons are likewise known (U.S. Patent 4,141,973 and U.S. Patent 5,409,904). Products which are at present in use have determined a great progress in eye surgery, for example in cataract operation.
- [0007] Particularly, the product commercially known as Healon® trade-mark, by Kabi Pharmacia, containing 1% of
- 25 hyaluronic acid having molecular weight of about 4,000,000 Daltons and a viscosity of about 200 cps which appears to be the most used product in the world, has ideal rheologic characteristics for eye microviscosurgery. Nevertheless, it presents the serious inconvenience that it has to be removed from the eye after operation. As a matter of fact, Healon® which remains in the eye causes pathologic elevation in P.I.O. (Shubert et al., 1984, Exp. Eye Res., 39, 137-152) and just a slight defence of endothelium.
- 30 [0008] Nevertheless, removal of Healon® from the eye performed by the surgeon after the operation is not likely to be complete and Healon® traces usually remain in the eye even after removal, with the result of evident consequences for the patient.
- [0009] Phacoemulsification has recently become the most used technique as for cataract operation. Advantages of such technique compared to techniques previously used can be summarized in a smaller incision, shorter time required
- 35 for the operation and a more rapid rehabilitation of the patient. Nevertheless, this technique doesn't permit to cancel the postoperative elevation in P.I.O., in both cases in which Healon® or one of the other substances known in Literature are used as viscoelastic substances, and it might cause complications to the corneal endothelium, owing to manipulation performed by the surgeon in the anterior chamber of the patient's eye.
- [0010] Injuries to the corneal endothelium caused by phacoemulsification have been related to mechanical trauma
- 40 caused by surgical manipulation and by irrigation of the eye with salt solutions.
- [0011] Therefore, it would be important to provide a novel composition able to eliminate the dangerous inconveniences which can result from small amounts of Healon® which remain in the eye, besides those which can result from inconveniences owing to mechanical removal of Healon® from the eye which has been operated upon, immediately after operation.

45 Summary of the Invention

- [0012] The author of the present invention surprisingly found an ophthalmic formulation, comprising hyaluronic acid, or a salt thereof, a citric acid salt, typically tri-sodium citrate, an antioxidant tolerated by the intraocular tissues and a
- 50 phosphate buffer, which has such characteristics to reduce the toxic effects owing to the operation and which can be left "in situ" without causing any significant elevation in P.I.O.
- [0013] Particularly, the present invention relates to a pharmaceutical formulation having a viscosity of 18-36 Pa-s (18,000 - 36,000 cps) at 2 sec<sup>-1</sup> and at 25°C, and comprising hyaluronic acid having molecular weight of 1,000,000 - 2,000,000 Daltons and at the concentration of 1.5% - 3.5% weight by volume, an ascorbic acid salt tolerated by the
- 55 intraocular tissues, citrate at the concentration of 0.01% - 0.05% weight by volume and phosphate buffer in order to maintain the formulation isotonic.

Description of Figures

[0014] Figure 1 shows a flow curve concerning the viscosity variation ( $\eta$ ) relative to the shear rate ( $\text{sec}^{-1}$ ) for the viscoelastic and non-Newtonian solution SVS18-(GII) and the salt solution (GIII).

[0015] Values of the shear rate ( $\text{sec}^{-1}$ ) are reported on abscissa and values of the viscosity variation expressed by  $\eta$  (mPa.s.) are reported on ordinate.

[0016] Figure 2 shows a flow curve of a solution according to the present invention: a) non-sonicated, without antioxidant; b) sonicated, without antioxidant; and c) sonicated, but having sodium ascorbate as antioxidant.

[0017] Values of the shear rate ( $\text{sec}^{-1}$ ) are reported on abscissa and the viscosity ( $\eta$ ) is reported on ordinate.

Detailed Description of the Invention

[0018] The present invention comprises a sodium hyaluronate solution in combination with a citric acid salt, typically tri-sodium citrate, an ascorbic acid salt tolerated by the intraocular tissues in isotonic salt solution buffered at neutral pH.

[0019] According to a typically embodiment of the present invention, the hyaluronic acid salt may be the only viscosity thickener included in the present formulations.

[0020] The method of preparation of the solution object of the present invention requires mixing of the components under aseptic conditions, by means of techniques and equipments usual for ophthalmic preparations.

[0021] The resulting formulation has a viscosity of 18-41 Pa.s (18,000 - 41,000 cps) (cps = centipoises) at  $2 \text{ sec}^{-1}$ , at  $25^\circ\text{C}$ .

[0022] Extractive (e.g., from cock comb, umbilical cord, etc.) or fermentative (e.g., from *Streptococcus*, etc.) NaHA has a molecular weight of 1,000,000 - 2,000,000 Daltons and a concentration of 15 - 35 mg/ml, which produces a preparation having a viscosity of 18-41 Pa.s (18,000 - 41,000 cps) at  $2 \text{ sec}^{-1}$  and at  $25^\circ\text{C}$ , preparation which maintains its own rheologic characteristics even in presence of substances such as sodium citrate, an antioxidant, e.g. sodium ascorbate and/or glucose, phosphate buffer and, if that is the case, sodium chloride, potassium chloride, magnesium chloride and calcium chloride. According to a preferred embodiment, the present formulations have a viscosity of 18 to 41 Pa.s (18,000 to 41,000 cps) at a share rate of  $2 \text{ sec}^{-1}$ , at  $25^\circ\text{C}$ , and of 0.55 to 1.25 Pa.s (550 to 1,250 cps) at a share rate of  $300 \text{ sec}^{-1}$ , at  $25^\circ\text{C}$ , corresponding to a viscosity versus share variation index (ratio of  $\eta$  at  $1 \text{ sec}^{-1}$  /  $\eta$  at  $300 \text{ sec}^{-1}$ ) of about 33. In the present formulation, the citrate will be at the concentration of 0.01% - 0.05%, preferably 0.03% weight by volume.

[0023] As antioxidant tolerated by the intraocular tissues, it will be possible to use at least one of the antioxidants selected from the group comprising glucose, sulphides, superoxide dismutase (SOD), cysteine and derivatives thereof. Furthermore, other antioxidants, tolerated by the intraocular tissues, known in literature, can be used, e.g. hydrosoluble antioxidants, antioxidants which have at least one -SH or -CHO group, peptides and enzymes.

[0024] Particularly, as antioxidant it will be possible to use sodium ascorbate at the concentration of 0.01% - 1%, preferably 0.02% weight by volume, and/or glucose at the concentration of 0.04% - 4%, preferably 0.07% weight by volume.

[0025] The applicant has unexpectedly found that antioxidants significantly reduce hyaluronic acid degradation due to sonication following pharmacoemulsification.

[0026] Typically, the present formulations contain an ascorbic acid salt, such as sodium ascorbate, as the antioxidant, preferably in association with another antioxidant.

[0027] Typical formulations according to the present invention contain both ascorbate and glucose.

[0028] As for phosphate salts based buffer, this is added in an amount sufficient to produce a neutral isotonic aqueous solution (e.g. pH 7.0 - 7.4, preferably pH 7.2).

[0029] According to a particular embodiment, besides the elements mentioned above, the formulation according to the present invention may comprise an isotonic salt solution comprising sodium chloride, potassium chloride, magnesium chloride, calcium chloride and dibasic sodium phosphate.

[0030] Therefore, the formulation according to the present invention may have the following composition:

- |                                                        |                |
|--------------------------------------------------------|----------------|
| - NaHA (M.W. 1,000,000 - 2,000,000 Daltons);           | 1.5 - 3.5%     |
| - $\text{Na}_3\text{Citrate}$                          | 0.01 - 0.05%   |
| - Sodium ascorbate                                     | 0.01 - 1.0%    |
| - glucose                                              | 0.04 - 4.0%    |
| - $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ | 0.26 - 0.39%   |
| - NaCl                                                 | 0.54 - 0.81%   |
| - KCl                                                  | 0.15 - 0.23%   |
| - $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$            | 0.01 - 0.02%   |
| - $\text{CaCl}_2 \cdot \text{H}_2\text{O}$             | 0.007 - 0.011% |

- water up to 100 ml. ; pH 7.0 - 7.4

[0031] According to further preferred embodiments, ascorbate concentrations are of 0.01-0.03%; glucose concentrations are of 0.04-1.00%; NaCl concentrations are of 0.60-0.70%.

[0032] According to a further preferred embodiment, the formulation according to the present invention may have the following composition:

- NaHA (M.W. 1,000,000 - 2,000,000 Daltons); 2.5%
- Na<sub>3</sub>citrate 0.03%
- Sodium ascorbate 0.02%
- glucose 0.07%
- Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 0.32%
- NaCl 0.68%
- KCl 0.19%
- MgCl<sub>2</sub>·6H<sub>2</sub>O 0.014%
- CaCl<sub>2</sub>·H<sub>2</sub>O 0.009%
- water up to 100 ml. ; pH 7.2

[0033] Unless otherwise stated, in the present text the % amounts of the components correspond to weight/volume %.

[0034] The aim of the present invention is also achieved using hyaluronic acid salts other than the sodium salt, as well as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup> and Mg<sup>++</sup> salts different from those hereinabove mentioned, preferred composition containing in addition to 1.5-3.5% weight/volume of a hyaluronic acid salt, the following amounts of ionic species and of glucose: Na<sup>+</sup> 100-220 mmol/l, and more preferably 100-150 mmol/l (not included that coming from NaHA); K<sup>+</sup> 20-30 mmol/l, typically about 25 mmol/l; Ca<sup>++</sup> 0.50-0.90 mmol/l, typically about 0.7 mmol/l; Mg<sup>++</sup> 0.40-1.00 mmol/l, typically about 0.7 mmol/l; Cl<sup>-</sup> 100-180 mmol/l, more preferably about 140-150 mmol/l; HPO<sub>4</sub><sup>=</sup> 7.0 - 11.00 mmol/l, typically about 9 mmol/l; citrate 0.30-2.00 mmol/l, typically 0.4-1.2 mmol/l; ascorbate 0.50-55.00 mmol/l, typically 0.50-1.00 mmol/l; glucose 2.0-225.00 mmol/l, typically 2.2-5.0 mmol/l.

[0035] The formulation according to the present invention has been used in the ophthalmic surgery in general. Particularly, in the ophthalmic surgery of the anterior segment (cataract, glaucoma, corneal or conjunctival pathology, etc.) or of the posterior segment (vitrectomy, detachment of retina, etc.) and it proved to be extremely efficient, particularly during cataract operation and more particularly during utilization of phacoemulsification.

[0036] The formulation according to the present invention appears to fulfil requirements of viscoelasticity and non-Newtonian behaviour necessary to the ophthalmic surgery, particularly to the cataract operation, either in presence or in absence of phacoemulsification. Furthermore, such formulation shows remarkable advantages when compared to the preparations on the market used in the eye surgery, that are Healon® (Kabi Pharmacia) or Viscoat® (Alcon):

- a lower influence on P.I.O.;
- it can be left "in situ" after the operation, instead of being removed; therefore, it avoids the remaining of residual traces, even after removal from the eye;
- a lower inflammatory effect (phlogosis);
- a lower loss of corneal endothelium cells, even when measured just 3 months after the operation.

[0037] Now, the present invention will be disclosed in the following example, according to a particular embodiment and compared to a solution known by the Prior Art.

[0038] The observation that the present formulations are endowed with good non-Newtonian properties, is in particular unexpected in view of the teaching of UPS 5,106,615, with reports that amounts of NaCl above 0.01% by weight (i.e. above 1.75 mmoles/l) destroy the non-Newtonian behaviour of hyaluronate based solutions, and that Ca<sup>++</sup> and other bivalent cations are even more efficient, as well as data showing that 25 mmoles/l of NaCl abolish such non-Newtonian behaviour.

[0039] Viscoelasticity is a rheological property possessed by materials which are viscous, but also exhibit elastic deformation when stressed (Remington's Pharmaceutical Science, Mack Publishing Company, 18th Ed., pages 319-320), whereas non-Newtonian behaviour is a rheological property, possessed by materials the viscosity of which varies in a manner not proportional to the applied shear stress (McGraw Hill Dictionary of Scientific Technical Terms, pages 479, 1010, 1020, 1601). So the two aforementioned properties refer to different rheological aspects, which may be concurrently present in some materials, such as for instance in hyaluronate based solutions substantially free from salts.

Example 1**Comparative test for phacoemulsification**

5 [0040] The surprising efficacy of the formulation according to the present invention was examined in a clinical study with 60 patients with cataract, patients who were assigned to 3 groups (indicated as GI, GII and GIII) each of 20 members, operated by means of phacoemulsification technique described and standardized hereinafter:

- scleral incision (350 microns deep) parallel to the limbus (1-2 mm behind this one);
- 10 - preparation of the scleral tunnel by means of lancet;
- aperture of the anterior chamber (by means of 3.2 mm callipered (calibrated) blade with inclination < 45°;
- input of the viscoelastic solution into the anterior chamber (total replacement of the aqueous humour);
- capsulorrhexis, hydrodissection and nucleodelineation;
- phacoemulsification (cross technique);
- 15 - aspiration of the masses and input of the viscoelastic solution into the capsular sac;
- implantation of 5 mm intracapsular eye lens;
- aspiration of the Healon® (GI), while the formulation according to the present invention (GII) and the solution of control (GIII) remain "in situ";
- apposition of a suture, if that is the case;

20 [0041] The average time of ultrasound used in the phacoemulsification is of 60 seconds +/- 15 seconds. The viscoelastic preparations used in this test were:

a) Healon®, whose composition is the following one:

25

HA (M.W. 4,000,000 Daltons)	1.0 g
NaCl	0.85 g
Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O	0.028 g
NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	0.004 g
and water for injectable preparations for the GI;	up to 100 ml,

30

b) a formulation according to a particular embodiment of the present invention having the following composition (indicated as SVS 18):

35

- Hyaluronic acid (M.W. 1,350,000 Daltons); 2.5 g
- Na<sub>3</sub>citrate 0.031 g
- Sodium ascorbate 0.022 g
- 40 - glucose 0.070 g
- Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O 0.322 g
- NaCl 0.678 g
- KCl 0.194 g
- MgCl<sub>2</sub>.6H<sub>2</sub>O 0.014 g
- 45 - CaCl<sub>2</sub>.H<sub>2</sub>O 0.009 g
- water for injectable preparations for GII; and up to 100 ml. ; pH 7.2

c) a preparation of a control salt solution containing:

50

HA (M.W. 1,350,000 Daltons)	2.5 g
NaCl	0.850 g
Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O	0.322 g
NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	0.004 g
water for injectable preparations for the GIII;	up to 100 ml, pH 7.2

55

[0042] GI was constituted of 12 men and 8 women being the average age of 60 years (range 35 - 72), GII was constituted of 11 men and 9 women being the average age of 62 years (range 38 - 72) and GIII was constituted of 11 men and 9 women being the average age of 61 years (range 34 - 73).

[0043] All operations were performed by the same surgeon, who always used the same technique, the same amount of viscoelastic substance and who removed the Healon® after the operation, while he left "in situ" the formulation according to the present invention and the salt solution of control.

[0044] After the operation, all patients were treated with a subconjunctival injection of 6 mg of betamethasone and with a dexamethasone and tobramycin based collyrium.

[0045] The topic therapy was performed for 3 weeks every morning and it was associated with 1% tropicamide based collyrium.

[0046] Parameters which were considered in this study were: intra-ocular pressure (P.I.O.) evaluated by means of applanation tonometry the day before the operation and then after 6 hours, 12 hours, 1, 7, 30 and 60 days; it must be recalled to memory that physiologic value of intra-ocular pressure is < 2799.3 Pa (21 mmHg); the postoperative inflammation was valued after 1, 3, 7, 30 and 60 days after the operation and it was expressed by arbitrary units (0 = absent, + = slight, ++ = intermediate, +++ = intense); number and morphology of endothelial cells were evaluated by Zeiss specular endothelial microscopy before the operation and after 2 months.

[0047] Results are reported in Tables 1, 2 and 3, respectively. By observing such Tables 1, 2 and 3, it is possible to assert what follows:

i) the formulation according to the present invention (SVS 18 of GII) relative to the Healon® can be left "in situ" after the operation, which not only doesn't cause elevation in pressure (on the contrary, after 6 hours the group treated with Healon® that is GI, has an average pressure of 2799.3±933.1 Pa (21±7 mmHg) against 2399.4 ±799.8 Pa (18±6 mmHg) of GII) (Table 1), but it also explains the lower incidence of postoperative phlogosis during the first day after the operation (Table 2);

ii) the formulation according to the present invention is the viscoelastic substance which permits the lower loss of endothelial cells (-7% two months after the operation), while the group wherein Healon® was used (GI) did undergo a loss of 11% during the same period of 2 months (Table 3);

TABLE 1 - INTRA-OCULAR PRESSURE (mmHg WHEREIN 1mmHg=133.3 Pa)  
mean value ± Standard Deviation

PATIENTS	PRESSIONE		TIME					
	0	6h	12h	1g	3g	7g	30g	60g
GI (HEALON*)	14±4	21±7	19±5	18±3	18±4	16±2	18±2	15±4
GII (SVS 18)	15±2	18±6	18±6	17±4	16±3	15±2	15±3	16±4
GIII (SALT SOLUTION)	12±5	18±7	19±6	19±4	17±5	18±3	16±4	15±3

TABLE 2 - OCULAR PHLOGOSIS  
arbitrary unit (0=absent; +=slight; +=intermediate;  
+++=intense)

DEGREE OF PHLOGOSIS PATIENTS	TIME					
		1 day	3 days	7 days	30 days	60 days
GI (HEALON*)	0	0	0	12	20	20
	+	9	15	8	0	0
	++	11	5	0	0	0
	+++	0	0	0	0	0
GII (SVS 18)	0	0	0	18	20	20
	+	14	16	2	0	0
	++	6	4	0	0	0
	+++	0	0	0	0	0
GIII (SALT SOLUTION)	0	1	2	16	19	20
	+	11	12	4	1	0
	++	9	6	0	0	0
	+++	0	0	0	0	0

TABLE 3 - ENDOTHELIAL CELLS DENSITY  
mean value  $\pm$  Standard Deviation

PATIENTS	PERIOD	PRE-OPERATIVE	60 days
GI (HEALON*)		2650 $\pm$ 450	2359 $\pm$ 425
GII (SVS 18)		2780 $\pm$ 333	2594 $\pm$ 370
GIII (SALT SOLUTION)		2690 $\pm$ 380	2448 $\pm$ 390

iii) the difference on endothelial cells between the two GII and GIII groups treated with SVS 18 and with the control salt solution respectively, is a clear evidence of the favourable effect of the formulation according to the present invention comprising the citrate and at least one antioxidant (as for the present case, sodium ascorbate and glucose) with respect to the one wherein sodium hyaluronate at the same concentration and having the same molecular weight is in buffered salt solution, only (Table 3).

[0048] By observing Figure 1, it can be noted that the SVS18 solution has a viscosity versus share rate variation index (ratio of  $\eta$  1 sec<sup>-1</sup>/ $\eta$  300 sec<sup>-1</sup>) of 33.6, while the same value is of 24.6 for the salt solution (GIII). The result is that the SVS18 has a viscosity versus share rate variation index of +27% with respect to the salt solution.

[0049] For the purpose of its utilization in ophthalmic surgery, in particular in cataract operation, the most important rheologic characteristic is the viscosity versus shear rate variation index, considered as ratio between two viscosity (low shear rate viscosity, non-moving fluid and high shear rate viscosity, fluid under mechanical stress).

[0050] The viscosity versus shear rate variation index is a measure of the versatility of a non-Newtonian substance, wherein the higher the index is, the more versatile the non-Newtonian fluid is from a rheologic point of view and therefore it will show a better behaviour during the operation.

[0051] The increase of +27% of the index, reported in Figure 1, confirms the fact that the SVS18 solution has rheologic characteristics more suitable for the needs of the operation exactly because of its peculiar formulation, making it superior over the salt solution.

Example 2**Effect of the antioxidants on sonicated formulations**

5 [0052] The effect of stabilization of the degradation of a sodium hyaluronate sonicated solution with a phacoemulsifier has been evaluated by means of addition of antioxidants.

[0053] Different formulations were prepared. The first one corresponded to the one reported in Example 1, but without antioxidants (neither glucose, nor ascorbate), while the other ones corresponded to the previous formulation, but comprising ascorbic acid at 0.02% as well as glucose 0.07% weight/volume.

10 [0054] Such solutions were sonicated for 5 minutes with a Alcon mod. Universal phacoemulsifier. The degradation of the sodium hyaluronate solution was monitored by means of viscosimetric measures performed before and after sonication with a BOHLIN VOR viscosimeter (Bohlin AG).

[0055] The results which were obtained are shown in Figure 2, wherein a flow curve of a solution according to the present invention a) non-sonicated, without antioxidant; b) sonicated, without antioxidant; and c) sonicated, but having sodium ascorbate as antioxidant, is reported.

15 [0056] By observing the flow curves showed in Figure 2, which were obtained for the formulation containing sodium ascorbate, a higher viscosity and a stabilization of about 20% relative to the sonicated solution without sodium ascorbate were observed.

20 [0057] Furthermore, the present invention relates to the use of an aqueous sodium hyaluronate solution, having molecular weight of 1,000,000 - 2,000,000 Daltons and a concentration of 1.5% - 3.5% weight by volume in combination with 0.01% - 0.05% weight by volume of citrate, and at least one antioxidant tolerated by the intraocular tissues and a phosphate buffer for the preparation of an ophthalmic formulation, where the obtained ophthalmic formulation has a viscosity of 18-41 Pa·s (18,000 - 41,000 cps) at 2 sec<sup>-1</sup> at 25°C.

[0058] Furthermore, the ophthalmic formulation according to the present invention is for the use in ophthalmic surgery.

25 [0059] Said ophthalmic surgery is for instance of the anterior segment (cataract operation, in particular by means of phacoemulsification, glaucoma operation, in particular by means of trabeculectomy, corneal operation, in particular keratoplasty, or conjunctival pathology etc.) or posterior segment (vitrectomy detachment of retina).

### 30 Claims

1. Ophthalmic formulation comprising 1.5%-3.5% weight by volume of hyaluronic acid having molecular weight of 1,000,000 - 2,000,000 Daltons or a salt thereof as a viscosity thickener, a citric acid salt, an ascorbic acid salt tolerated by the intraocular tissues and a phosphate buffer.
- 35 2. Formulation according to claim 1, having a viscosity of 18-41 Pa·s (18,000 - 41,000 cps) at 2 sec<sup>-1</sup> at 25°C.
3. Formulation according to claim 1, also comprising at least one antioxidant selected from the group comprising glucose, cysteine and derivatives thereof, sulphides, superoxide dismutase, hydrosoluble antioxidants, antioxidants having at least one -SH or -CHO group, peptides and enzymes.
- 40 4. Formulation according to claim 3, wherein said antioxidant is glucose.
5. Formulation according to claim 1, wherein said ascorbic acid salt is sodium ascorbate.
- 45 6. Formulation according to claim 4, wherein glucose is at the concentration of 0.04% - 4% weight by volume.
7. Formulation according to claim 1, containing both ascorbate and glucose.
- 50 8. Formulation according to claim 1, wherein the citrate is at the concentration of 0.01% - 0.05% weight by volume.
9. Formulation according to claim 1, wherein the phosphate buffer is added in amount sufficient to produce an isotonic aqueous solution.
- 55 10. Formulation according to claim 1, also comprising an isotonic salt solution comprising sodium chloride, potassium chloride, magnesium chloride, calcium chloride and dibasic sodium phosphate.
11. Ophthalmic formulation according to claim 1, having the following composition:



- sodium hyaluronate (m.w. 1,000,000 - 2,000,000 Daltons); 1.5 - 3.5%
- Na<sub>3</sub>citrate 0.01 - 0.05%
- Sodium ascorbate 0.01 - 1.0%
- glucose 0.04 - 4.0%
- Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 0.26 - 0.39%
- NaCl 0.54 - 0.81%
- KCl 0.15 - 0.23%
- MgI<sub>2</sub>·6H<sub>2</sub>O 0.01 - 0.02%
- CaCl<sub>2</sub>·H<sub>2</sub>O 0.007 - 0.011%
- water up to 100 ml. ; pH 7.0 - 7.4

12. Ophthalmic formulation according to claim 11, having the following composition:

- sodium hyaluronate (m.w. 1,350,000 Daltons); 2.5%
- Na<sub>3</sub>citrate 0.03%
- Sodium ascorbate 0.02%
- glucose 0.07%
- Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 0.32%
- NaCl 0.68%
- KCl 0.19%
- MgCl<sub>2</sub>·6H<sub>2</sub>O 0.014%
- CaCl<sub>2</sub>·H<sub>2</sub>O 0.009%
- water up to 100 ml. ; pH 7.2

13. Formulation according to claim 1, containing hyaluronic acid as the only viscosity thickener.

14. Formulation according to claim 1, containing: Na<sup>+</sup> 100-220 mmol/l; K<sup>+</sup> 20-30 mmol/l; Ca<sup>++</sup> 0.50-0.90 mmol/l; Mg<sup>++</sup> 0.40-1.00 mmol/l; Cl<sup>-</sup> 100-180 mmol/l; HPO<sub>4</sub> = 7.0 - 11.00 mmol/l; citrate 0.30-2.00 mmol/l; ascorbate 0.50-55.00 mmol/l; glucose 2.0-225.00 mmol/l.

15. Use of an aqueous solution containing hyaluronic acid having molecular weight of 1,000,000 - 2,000,000 Daltons or a salt thereof, at a concentration of 1.5% - 3.5% weight by volume, in combination with citrate, an ascorbic acid salt tolerated by the intraocular tissues and a phosphate buffer for the preparation of an ophthalmic formulation.

16. Use according to claim 15, also comprising an isotonic salt solution comprising sodium chloride, potassium chloride, magnesium chloride, calcium chloride and dibasic sodium phosphate.

17. Use according to claim 15 wherein the obtained ophthalmic formulation has a viscosity of 18-41 Pa.s (18,000-41,000 cps) at 2 sec<sup>-1</sup> at 25°C.

18. Ophthalmic formulation according to claim 1, for use in ophthalmic surgery.

19. Formulation according to claim 5, wherein sodium ascorbate is at concentration of 0.01%-100% weight by volume.

#### Patentansprüche

1. Ophthalmische Formulierung, umfassen 1,5 bis 3,5 Gew.% pro Volumen Hyaluronsäure mit einem Molekulargewicht von 1.000.000 bis 2.000.000 Dalton oder ein Salz davon als Viskositätsverdicker, ein Zitronensäuresalz, ein durch das intraokulare Gewebe toleriertes Ascorbinsäuresalz und einen Phosphatpuffer.
2. Formulierung gemäss Anspruch 1, mit einer Viskosität von 18 bis 41 Pa.s (18.000 bis 41.000 cps) bei 2 sec<sup>-1</sup> und 25°C.
3. Formulierung gemäss Anspruch 1, weiterhin umfassend mindestens ein Antioxidanz ausgewählt aus der Gruppe, umfassend Glukose, Cystein und Derivate davon, Sulphide, Superoxiddismutase, wasserlösliche Antioxidantien, Antioxidantien mit mindestens einer - SH- oder -CHO-Gruppe, Peptide und Enzyme.

4. Formulierung gemäss Anspruch 3, wobei das Antioxidanz Glukose ist.
5. Formulierung gemäss Anspruch 1, wobei das Ascorbinsäuresalz Natriumascorbat ist.
- 5 6. Formulierung gemäss Anspruch 4, wobei Glukose eine Konzentration von 0,04 bis 4 Gew.% pro Volumen hat.
7. Formulierung gemäss Anspruch 1, enthaltend Ascorbat und Glukose.
8. Formulierung gemäss Anspruch 1, wobei das Citrat eine Konzentration von 0,01 bis 0,05 Gew.% pro Volumen hat.
- 10 9. Formulierung gemäss Anspruch 1, wobei der Phosphatpuffer in einer ausreichenden Menge zugegeben wird, um eine isotonische wässrige Lösung herzustellen.
- 15 10. Formulierung gemäss Anspruch 1, weiterhin umfassend eine isotonische Salzlösung umfassend Natriumchlorid, Kaliumchlorid, Magnesiumchlorid, Kalziumchlorid und zweibasiches Natriumphosphat.
11. Ophthalmische Formulierung gemäss Anspruch 1, mit der folgenden Zusammensetzung:
  - Natriumhyaluronat (M.W. 1.000.000 - 2.000.000 1,5 - 3,5 % Dalton)
  - 20 - Na<sub>3</sub>-Citrat 0,01 - 0,05 %
  - Natriumascorbat 0,01 - 1,0 %
  - Glukose 0,04 - 4,0 %
  - Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 0,26 - 0,39 %
  - NaCl 0,54 - 0,81 %
  - 25 - KCl 0,15 - 0,23 %
  - MgCl<sub>2</sub>·6H<sub>2</sub>O 0,01 - 0,02 %
  - CaCl<sub>2</sub>·H<sub>2</sub>O 0,007 - 0,011 %
  - Wasser auf 100 ml; pH 7,0 - 7,4
- 30 12. Ophthalmische Formulierung gemäss Anspruch 1, mit der folgenden Zusammensetzung:
  - Natriumhyaluronat (M.W. 1.000.000 - 2.000.000 Dalton) 2,5 %
  - Na<sub>3</sub>-Citrat 0,03 %
  - Natriumascorbat 0,02 %
  - 35 - Glukose 0,07 %
  - Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 0,32 %
  - NaCl 0,68 %
  - KCl 0,19 %
  - MgCl<sub>2</sub>·6H<sub>2</sub>O 0,014 %
  - 40 - CaCl<sub>2</sub>·H<sub>2</sub>O 0,009 %
  - Wasser auf 100 ml; pH 7,2
13. Formulierung gemäss Anspruch 1, enthaltend Hyaluronsäure als einziges Viskositätsverdickungsmittel.
- 45 14. Formulierung gemäss Anspruch 1, enthaltend: Na<sup>+</sup> 100 bis 220 mmol/l; K<sup>+</sup> 20 bis 30 mmol/l; Ca<sup>++</sup> 0,50 bis 0,90 mmol/l; Mg<sup>++</sup> 0,40 bis 1,00 mmol/l; Cl<sup>-</sup> 100 bis 180 mmol/l; HPO<sub>4</sub> = 7,0 bis 11,00 mmol/l; Citrat 0,30 bis 2,00 mmol/l; Ascorbat 0,50 bis 55,00 mmol/l; Glukose 2,0 bis 225,00 mmol/l.
- 50 15. Verwendung einer wässrigen Lösung, enthaltend Hyaluronsäure mit einem Molekulargewicht von 1.000.000 bis 2.000.000 Dalton oder ein Salz davon mit einer Konzentration von 1,5 bis 2,5 Gew.% pro Volumen in Kombination mit Citrat, durch das innere Augengewebe toleriertes Ascorbinsäuresalz und ein Phosphatpuffer zur Herstellung einer ophthalmischen Formulierung.
16. Verwendung gemäss Anspruch 15, weiterhin umfassend eine isotonische Salzlösung umfassend Natriumchlorid, Kaliumchlorid, Magnesiumchlorid, Kalziumchlorid und zweibasiches Natriumphosphat.
- 55 17. Verwendung gemäss Anspruch 15, wobei die erhaltene ophthalmische Formulierung eine Viskosität von 18 bis 41 Pa·s (18.000 bis 41.000 cps) bei 2 sec<sup>-1</sup> und 25°C aufweist.

18. Ophthalmische Formulierung gemäss Anspruch 1 zur Verwendung in der ophthalmischen Chirurgie.

19. Formulierung gemäss Anspruch 5, wobei das Natriumascorbat eine Konzentration von 0,01 bis 1,00 Gew.% pro Volumen hat.

# Revendications

1. Formulation ophtalmique comprenant de 1,5 % à 3,5 % en poids par volume d'acide hyaluronique ayant un poids moléculaire de 1 000 000 à 2 000 000 Daltons ou un sel de celui-ci en tant qu'agent épaississant de viscosité, un sel d'acide citrique, un sel d'acide ascorbique toléré par les tissus intraoculaires et un tampon phosphate.

2. Formulation selon la revendication 1, ayant une viscosité de 18 à 41 Pa.s (18 000 à 41 000 cps) à 2 s<sup>-1</sup> et 25°C.

3. Formulation selon la revendication 1, comprenant également au moins un antioxydant choisi dans le groupe comprenant le glucose, la cystéine et des dérivés de celle-ci, des sulfures, la superoxyde dismutase, des antioxydants hydrosolubles, des antioxydants comprenant au moins un groupe -SH ou -CHO, des peptides et des enzymes.

4. Formulation selon la revendication 3, dans laquelle ledit antioxydant est le glucose.

5. Formulation selon la revendication 1, dans laquelle ledit sel d'acide ascorbique est l'ascorbate de sodium.

6. Formulation selon la revendication 4, dans laquelle le glucose est à une concentration de 0,04 % à 4 % en poids par volume.

7. Formulation selon la revendication 1, contenant à la fois de l'ascorbate et du glucose.

8. Formulation selon la revendication 1, dans laquelle le citrate est à la concentration de 0,01 % à 0,05 % en poids par volume.

9. Formulation selon la revendication 1, dans laquelle le tampon phosphate est ajouté en quantité suffisante pour produire une solution aqueuse isotonique.

10. Formulation selon la revendication 1, comprenant également une solution de sel isotonique comprenant du chlorure de sodium, du chlorure de potassium, du chlorure de magnésium, du chlorure de calcium et du phosphate de sodium dibasique.

11. Formulation ophtalmique selon la revendication 1, ayant la composition suivante :

- hyaluronate de sodium (p.m. 1 000 000 à 2 000 000 Daltons) ; 1,5 à 3,5 %
- Na<sub>3</sub>citrate 0,01 à 0,05 %
- ascorbate de sodium 0,01 à 1,0 %
- glucose 0,04 à 4,0 %
- Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O 0,26 à 0,39 %
- NaCl 0,54 à 0,81 %
- KCl 0,15 à 0,23 %
- MgCl<sub>2</sub>.6H<sub>2</sub>O 0,01 à 0,02 %
- CaCl<sub>2</sub>.H<sub>2</sub>O 0,007 à 0,011 %
- eau q.s.p. 100 ml ; pH 7,0 à 7,4

12. Formulation ophtalmique selon la revendication 11, ayant la composition suivante :

- hyaluronate de sodium (p.m. 1 350 000 Daltons) ; 2,5 %
- Na<sub>3</sub>citrate 0,03 %
- ascorbate de sodium 0,02 %
- glucose 0,07 %
- Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O 0,32 %
- NaCl 0,68 %

- KCl 0,19 %
- $MgCl_2 \cdot 6H_2O$  0,014 %
- $CaCl_2 \cdot H_2O$  0,009 %
- eau q.s.p. 100 ml ; pH 7,2

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13. Formulation selon la revendication 1, contenant de l'acide hyaluronique en tant qu'agent épaississant de viscosité.

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14. Formulation selon la revendication 1, contenant :  $Na^+$  100 à 220 mmol/l ;  $K^+$  20 à 30 mmol/l ;  $Ca^{++}$  0,50 à 0,90 mmol/l ;  $Mg^{++}$  0,40 à 1,00 mmol/l ;  $Cl^-$  100 à 180 mmol/l ;  $HPO_4=$  7,0 à 11,00 mmol/l ; citrate 0,30 à 2,00 mmol/l ; ascorbate 0,50 à 55,00 mmol/l ; glucose 2,0 à 225,00 mmol/l.

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15. Utilisation d'une solution aqueuse contenant de l'acide hyaluronique ayant un poids moléculaire de 1 000 000 à 2 000 000 Daltons ou un sel de celui-ci, à une concentration de 1,5 % à 3,5 % en poids par volume, en association avec un citrate, un sel d'acide ascorbique toléré par les tissus intraoculaires et un tampon phosphate pour la préparation d'une formulation ophtalmique.

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16. Utilisation selon la revendication 15, comprenant également une solution de sel isotonique comprenant du chlorure de sodium, du chlorure de potassium, du chlorure de magnésium, du chlorure de calcium et du phosphate de sodium dibasique.

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17. Utilisation selon la revendication 15, dans laquelle la formulation ophtalmique obtenue a une viscosité de 18 à 41 Pa.s (18 000 à 41 000 cps) à  $2 s^{-1}$  et 25°C.

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18. Formulation ophtalmique selon la revendication 1, pour utilisation en chirurgie ophtalmique.

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19. Formulation selon la revendication 5, dans laquelle l'ascorbate de sodium est à une concentration de 0,01 % à 1,00 % en poids par volume.

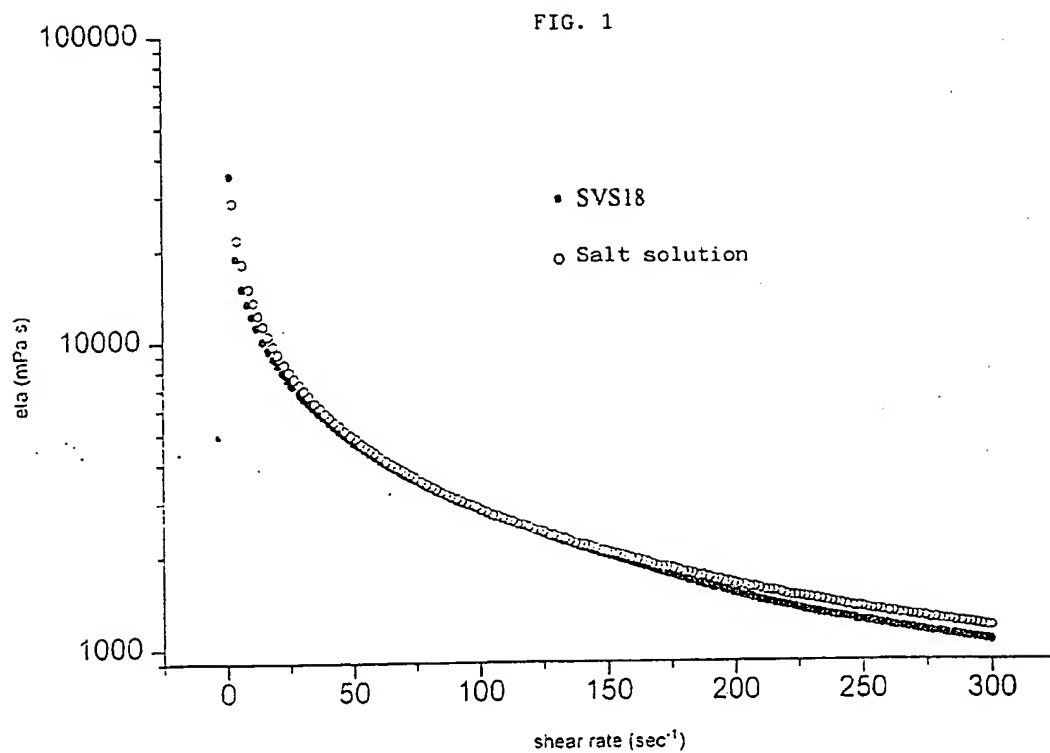


FIG. 2

